

TABLE 1

| Curve Number | U.A.-Identification | Fibrinogen Equivalent | mg/ml |
|--|---|-----------------------|--------|
| Sample size (0.2 ml, 2 μ l of 1/10 dilution. | | | |
| 1. (calibration) | 0.25 Mg. Rabbit IgG | 0.32 | Calib. |
| 2. | 10 ⁷ (Fibrinogen-Fibrin-Polymer) | 0.33 | 0.16 |
| 3. | 700,000 (Fibrinogen dimer) | 0.036 | 0.18 |
| 4. | 340,000 (Fibrinogen dimer) | 0.35 | 1.75 |
| 5. | 280,000 (Fragment X) | 0.027 | 0.14 |
| 6. | 40,000 to 30,000 (Fragment A, B or C) | 0.006 | 0.03 |
| 7. (calibration) | 0.5 Mg rabbit IgG | 0.16 | calib. |

EXAMPLE 3

A second method for attaching a separated protein to the novel gel of this invention has been demonstrated. In this method the conventional gel filtration methods for detection of fibrin complexes by plural fraction collection and multiple analyses of the eluted protein to characterize the chromatograph is superseded.

The proteins are separated by filtration through the glyoxal agarose gel of this invention separating them into displaced positions in the gel. Subsequently, the separated displaced proteins in the gel are immobilized into tight zones by electrophoresing alkaline cyanoborohydride into the suspending gel medium. Use of small disposable columns (Pasteur pipettes) for this zonal immobilization is advantageous. Thereafter, the immobilized zonal fractions are stained. A fluorescent or radio iodinated anti-human fibrinogen antibody is useful as the staining medium.

The procedural steps of Example 3 are illustrated in FIG. 3. An analysis is depicted based on the procedure in FIG. 4.

Referring specifically to FIG. 2 the following description is pertinent:

Cascade immunoelectrophoretic analysis of the molecular weight distribution of fibrinogen related antigens in the plasma fibrinogen sample. The plasma proteins together with a fluorescent labelled ribonuclease marker were separated by electrophoresis and subsequently immobilized in the sample gel. Carbamylated anti-fibrinogen antibody was electrophoresed into the sample gel and then removed by reversing current. Antibody that was retained by the immobilized fibrinogen antigens in the gel was then desorbed by electrophoresing sodium dodecyl sulfate into the gel and through a spacer gel in which the SDS was removed by precipitation with potassium ion. On continuing electrophoresis, the anti-fibrinogen antibody migrated through

the spacer without interference from SDS, and into a gel containing anti IgG antibodies for measurement of the anti-fibrinogen. The areas under the rockets formed by the IgG-anti IgG precipitate measures the amount of the variant forms of fibrinogen antigens in the sample gel. The quantities of antigens are expressed on the basis that 1 μ g of fibrinogen absorbs 1.6 μ g of anti-fibrinogen antibody. Peaks 1 and 7 in the illustration are from known amounts of anti-fibrinogen antibody applied as calibration standards. The molecular weight, concentrations, and probable nature of the fibrinogen derivatives in the sample are tabulated in Table 1.

Referring specifically to FIG. 4, fibrin complexes and fibrinogen in a human plasma sample were separated on a column of 4% glyoxyl agarose equilibrated with rabbit fibrinogen. The protein was then immobilized, and the distribution of fibrinogen related antigens was established with fluoresceinated anti-human fibrinogen antibody.

Having thus described the best mode presently known to me to prepare the novel gel of this invention and illustrated novel methods for its usefulness in the arts,

What I claim is:

1. A method of separation of a complex protein fraction(s) of biochemical origin from others of said complex proteins present in dilute aqueous solution which comprises selectively binding and retaining said protein fraction by selective zonal sorption by means of intimate contact of said liquid with a gel comprising glyoxal agarose.

2. A method of temporarily fixing an isolated selected complex protein fraction recovered by the method of claim 1 which comprises treating the sorbed fraction separated in the glyoxal agarose gel by increasing the pH of the gel phase to above about 9.5.

3. The method of desorbing isolated protein fraction(s) from glyoxal agarose sorbate of claim 1 which comprises electrophoresing sodium dodecyl sulfate into the sorbate glyoxal agarose gel thereby to displace and recover individual isolated protein fraction(s) therefrom.

4. The method of permanently immobilizing separated protein fractions isolated in accordance with claim 1 in a containant glyoxal agarose gel which comprises treating the said gel to increase the pH to above about 9.5 with an alkaline buffer solution containing sodium cyanoborohydride.

5. The method of claim 4, wherein the treatment of the sorbate glyoxal agarose gel containing the isolated protein fractions with the alkaline sodium cyanoborohydride buffer is by electrophoresis.

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